

METHODS AND COMPOSITIONS FOR THE TREATMENT OF PSORIASIS

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Patent Application No. 60/065,774, filed November 17, 1997. The priority application is hereby incorporated herein by reference in its entirety.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

[0002] This invention was made in part during work supported by a grant from the United States Public Health Service (CA72018). The government has certain rights in the invention.

BACKGROUND

[0003] Psoriasis is a chronic condition that affects as much as 2.6% of the population of the developed world. A recent survey reported by the National Psoriasis Foundation estimates that 6.4 million people suffers from psoriasis, of which about 500,000 is rated as severe. The annual patient cost for treating psoriasis is currently estimated at \$1.6 to \$3.2 billion. Every year, about 400 people are granted disability by the Social Security Administration, and another 400 people die from psoriasis-related causes.

Characteristics of Psoriasis

[0004] It is not known what causes psoriasis, although there is evidence of a genetic predisposition and an autoimmune etiology. Onset may be triggered by systemic infections such as strep throat, skin injury, vaccinations, and certain oral medications such as steroids.

Subsequently, the immune system is thought to induce inflammation and excessive skin cell reproduction, which can be exacerbated by additional factors such as stress and diet.

[0005] In normal skin, the time for a cell to move from the basal layer through the granular layer is 4-5 weeks. In psoriatic lesions, the time is decreased 7-10 fold because of a shortened cell cycle time, an increase in the absolute number of cells capable of proliferating, and an increased rate of division. T cell mediated immune responses appear to be responsible for the inflammation and hyperproliferation of keratinocytes. Neutrophils are found in psoriatic lesions, associated with increased levels of plasminogen activator. Psoriatic fibroblasts have increased levels of enzymes involved in collagen synthesis, secondary to expansion of the papillary dermis. Psoriatic plaques comprise HLA-DR positive keratinocytes and Langerhans cells, and activated T cells expressing elevated levels of IL-2 receptors.

[0006] The typical lesion of psoriasis is a well-demarcated erythematous plaque, covered by thick, silvery scales. Psoriasis can become so extensive as to cause exfoliative erythroderma, in which the entire epidermal surface is in a state of hyperproliferation. Glutamate psoriasis is a form of the disease following streptococcal pharyngitis, with widely distributed characteristic 1-3 cm lesions. Pustular psoriasis is characterized by numerous sterile pustules of 2-5 mm in diameter, and may lead to an acute, explosive, life-threatening episode of fever, chills, leukocytosis, hypoalbuminemia, and hypocalcemia, demanding immediate, vigorous therapy. Previously stable plaque-type psoriasis can be acutely exacerbated by viral infections, particularly HIV. Psoriasis is also associated with five different forms of psoriatic arthritis, including distal interphalangeal involvement; an asymmetric, oligoarticular pattern; a symmetric polyarthritides; arthritis mutilans; and sacroiliitis and spondylitis.

[0007] The inflammation and hyperproliferation of psoriatic tissue is associated with a different histological and antigenic profile than normal skin. Dabelsteen et al. used a panel of anti-carbohydrate monoclonal antibodies to compare psoriatic tissue with the surrounding dermis. The glycosylation pattern in psoriatic epithelium is changed in two ways: some carbohydrates are expressed at an earlier stage of cell maturation. In addition, certain biosynthetic precursor antigens not expressed in normal skin were found in psoriatic skin.

[0008] Paller et al. (1989) investigated the distribution of ganglioside GM3 using an antibody designated 8G9D8. At the electron microscope level, antibody deposition was seen in the corneocyte envelope. Disposition was significantly decreased or absent in disorders of excessive keratinocyte proliferation, including squamous cell carcinomas, congenital ichthysiform erythrodermas, prokeratosis, and psoriasis. In a subsequent study, Paller et al. (1993) found that when GM3 was added to keratinocytes from normal foreskin, lesional skin from patients with psoriasis or ichthyosis, and to cutaneous squamous carcinoma lines, it inhibited growth in a dose-dependent manner at concentrations of 10-100 μ M. Confluent undifferentiated keratinocytes were least sensitive. The gangliosides GD3, 9-O-acetyl-GD2, and GD1b also inhibited keratinocyte proliferation. Gangliosides GM1 and GD1a, and sialic acid had little effect. The authors concluded that preferential activation of sialyltransferase II may be involved in control of keratinocyte growth, but not differentiation.

[0009] Concharenko et al. studied ganglioside expression on the erythrocytes and serum of healthy subjects, and patients with psoriasis. During phases of exacerbation, a marked decrease was observed in the content of GM2 and GM3 fractions of the red cells, and GD1a decreased in serum. The presence of a new monosialoganglioside fraction was noted during exacerbation, both in serum and on red cells. The ganglioside spectrum of patients in clinical remission of psoriasis was almost normal.

[0010] Heidenheim et al. describe a monoclonal antibody designated UM4D4 which recognizes the cell surface marker CDw60. This marker is present on a subset of normal T cells, melanocytes, malignant melanoma cells, and hyperproliferative psoriatic keratinocytes. CDw60 antibodies bind to the acetylated form of GD3. 74% of basal cell carcinomas express CDw60, whereas CDw50 expression in normal skin is confined to melanocytes and a few scattered keratinocytes at the basal cell layer. Skov et al. recently reported that in psoriatic skin, basal and suprabasal keratinocytes express CDw60. Cloned T cell lines obtained from lesional skin upon initiation were found to release a cocktail of soluble factors including IL-4 and IL-13, that up-regulated CDw60 expression on cultured normal keratinocytes.

Currently available treatments for psoriasis

[0011] Classical treatments of psoriasis include calcipotriene (a vitamin D₃ derivative), topical coal tar preparations, systemic antimitotic agents such as methotrexate, and retinoids, particularly etretinate. Extensive psoriasis can be treated by photosensitization with oral 8-methoxypsoralen, followed by ultraviolet A. Corticosteroids are given for psoriatic arthritis and acute attacks of pustular psoriasis. More recently, cyclosporin A has been tested in clinical trials at doses of 3–7 mg/kg with promising results, but associated with the risk of renal toxicity. There is no cure.

[0012] Current biotechnology approaches to psoriasis treatment relate to a direct pharmaceutical-mediated attack, either on cell proliferation or on the immune component of the disease. Japanese patent application JP 6145069 describes angiogenesis inhibitors comprising ganglioside GM3 or a GM3 analog as an active agent. At 100 µg/mL, GM3 showed growth of normal human antioendothelial cells of 4.5×10^4 on day 5, compared with 76×10^4 in controls. U.S. Patent No. 5,339,977 describes n-deacetyl-lysoganglioside derivatives for use as phospholipase A2 inhibitors for the treatment of proliferative and autoimmune diseases, including various forms of cancer, psoriasis, and rheumatoid arthritis.

[0013] An IL-2 fusion toxin has been developed (Seragen, Inc.) that is designed to selectively destroy activated T cells in psoriatic plaques, leaving normal cells alone. The objective is to destroy activated T cells, and thereby clear the psoriasis. A Phase II study has been performed in which test doses of 5, 10, and 15 µg/kg were administered per day. Comparable improvement was observed in patients with moderate to severe psoriasis. However, in order to obtain this response, the compound was administered three days per week for four weeks.

[0014] Various formulations containing the compound BCX-34 for psoriasis, cutaneous T cell lymphoma, and HIV infection have been tested (Bioworld Today, Sept. 29, 1997; see also WO 95/01355; WO 93/21187; WO 90/10631; U.S. Pat. Nos. 5,008,270, 5,008,265, and 4,985,434). BCX-34 is a small molecule drug that inhibits purine nucleoside phosphorylase, a human enzyme believed to be involved in the proliferation of T cells. An oral formulation is being tested in an ongoing Phase I/II trial. A topical formulation

advanced to the Phase III stage for both lymphoma and psoriasis. The Phase III psoriasis study showed only a 14% greater improvement in mean lesion scores in the treated group compared to placebos, which for these studies was not statistically significant.

[0015] Accordingly, there is a need for therapeutic compositions that are effective in managing psoriasis, particularly if they are effective when given on an occasional basis. The present invention relates to a strategy in which the patient's own immune system is recruited into an active role against the disease.

SUMMARY OF THE INVENTION

[0016] The invention provides methods of using compositions which elicit an immune response against an antigen that is aberrantly expressed in psoriatic tissue.

[0017] Accordingly, in one aspect, the invention provides methods for treating psoriasis in an individual, comprising administering a composition effective in stimulating a specific immunological response against an antigen aberrantly expressed in human psoriatic tissue. These composition(s) comprise an antigen that shares immunological characteristics of an antigen that is aberrantly expressed in psoriatic tissue (such as human psoriatic tissue). Antigens aberrantly expressed in psoriasis include but are not limited to gangliosides. While a detectable immunological response is likely to be beneficial, efficacy can also be deduced by an improvement in symptoms or control of the psoriatic condition beyond what would be expected without treatment.

[0018] Certain embodiments of the invention include methods for treating psoriasis in an individual administering by eliciting an anti-ganglioside immunological response in the subject. The immunological response can be elicited using any suitable immunogen and/or immunogenic composition, such as: (1) a ganglioside in an immunogenic form, such as GM2, GM3, GD1b, GD2, 9-O-acetyl GD2, GD3, GD3 lactone, 9-O-acetyl GD3, and GT3; (2) an anti-idiotypic for a ganglioside, such as the monoclonal antibodies 1A7, 4B5, or BEC-2; or (3) a polynucleotide encoding an anti-idiotypic for a ganglioside. The immunological response can have humoral or cellular components, and preferably has both.

[0019] Further embodiments of the invention relate to preparing a composition for use in the treatment of psoriasis by preparing a composition comprising the determinant in an immunogenic form, or else raising an anti-idiotypic antibody against a monoclonal antibody that binds the antigenic determinant. Also embodied are methods for screening a composition for efficacy in treating psoriasis, involving administering the composition to human subjects, and monitoring disease progression (optionally in combination with immunological parameters) following treatment.

[0020] A further embodiment of the invention is a composition containing a ganglioside, an anti-idiotypic antibody for a ganglioside, or a polynucleotide encoding a ganglioside, packaged with an indication of its suitability for use in treating psoriasis.

[0021] Further embodiments of the invention relate to the use of a component selected from the group consisting of a ganglioside, an anti-idiotypic antibody for a ganglioside, and an expression vector encoding an anti-idiotypic antibody for a ganglioside, in the manufacture of a medicament for the treatment of psoriasis.

BRIEF DESCRIPTION OF THE FIGURES

[0022] **Figure 1** is a scheme for evaluating the effect of raising an anti-GD2 immunological response using an anti-idiotypic vaccine to treat embodiments with severe chronic plaque psoriasis.

[0023] **Figure 2** is a depiction of the cDNA sequence (SEQ ID NO:1) and the amino acid sequence (SEQ ID NO:2) of the light chain variable region of 1A7 and adjoining residues.

[0024] **Figure 3** is a depiction of the cDNA sequence (SEQ ID NO:3) and the amino acid sequence (SEQ ID NO:4) of the heavy chain variable region of 1A7 and adjoining residues.

DETAILED DESCRIPTION

[0025] It is an object of this invention to provide compositions that are newly applied in the treatment of psoriasis in its various manifestations. Ideally, the compositions promote resolution of the clinical features, but stabilization of the condition is a satisfactory outcome.

It is desirable that the effect be enduring, so that once an effect is achieved, readministration of the composition, if any, need occur on only an occasional basis. To accomplish this, the usual mode is to recruit active participation of the immune system of the host to react against a target antigen on psoriatic tissue.

[0026] This invention is based in part on the discovery that patients immunized systemically with a suitable vaccine composition mount a response directed against a psoriasis-specific antigen. As illustrated in Example 3, the response includes circulating antibodies that specifically adhere to affected tissue. Without intending to be bound by theory, it may be that the immune response to the target antigen promotes elimination or metabolic down-regulation of cells bearing the target antigen, or that it interferes mechanistically with a pathological phenotype of affected cells (such as proliferation) that is mediated, at least in part, by the target antigen. As a result, the presence of the immune response promotes the clinical resolution of the condition.

[0027] Several target antigens are believed to be suitable as vaccine targets in psoriasis, and various types of vaccines can be used to obtain the desired result. Illustrative examples are given in the sections that follow.

Definitions

[0028] In reference to the therapeutic methods and compositions of this invention, the term "psoriasis" refers to all skin conditions in the clinical arts described by this term, and to psoriatic-associated conditions, including psoriatic arthritis.

[0029] "Psoriatic tissue" refers to tissue affected by psoriasis and affected cells contained within the tissue, but not to cells that have migrated to the site such as leukocytes. Preferably, the psoriatic tissue is from a human.

[0030] Particular molecules referred to in this disclosure, such as CEA, gangliosides designated GM1, GM2, and so on, are meant to include not only the intact molecule, but also allotypic and synthetic variants, synthetic analogs, fusion molecules, conjugates, and other derivatives that contain the parent molecule and its fragments that are recognized by antibodies specific for the intact molecule.

[0031] An “effective amount” is an amount sufficient to effect a beneficial or desired clinical result. An effective amount can be administered in one or more doses. For purposes of this invention, an effective amount of ganglioside, antibody, or other composition is an amount that induces an immune response against a psoriasis antigen.

[0032] “Immunological activity” of a particular immunogen or vaccine component refers to the ability to raise an immune response. A specific immune response may comprise antibody, B cells, T cells, and any combination thereof, and effector functions resulting therefrom. Included are the antibody-mediated functions ADCC and complement-mediated cytotoxicity (CMC). A T cell response can include T helper cell function, cytotoxic T cell function, or inflammation/inducer T cell function. A compound or composition able to elicit a specific immune response according to any of these criteria is referred to as “immunogenic”.

[0033] An antigen that “shares immunological characteristics” with another antigen is an antigen that, when administered in appropriate form (such as, for example, alone, in conjunction with an adjuvant, in association with (or conjugated to) a compound), elicits an immunological activity. For purposes of this invention, it is also understood that an antigen comprises an antigenic determinant(s) (as is well understood in the art).

[0034] A “polynucleotide” is a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and nucleotide analogs in any combination. A “vector” refers to a recombinant DNA or RNA plasmid or virus that comprises a heterologous polynucleotide to be delivered into a target cell, either in vitro or in vivo.

[0035] The terms “polypeptide”, “peptide” and “protein” are used interchangeably to refer to polymers of amino acids of any length, and may be interrupted by non-amino acids.

[0036] An “aberrantly expressed antigen” is an antigen that is uniquely expressed, overexpressed, and/or underexpressed in conjunction with a disease state. For purposes of this invention, the disease state is psoriasis.

[0037] An “immunogenic form” of an antigen is a form of or formulation comprising the antigen which renders the antigen immunogenic. Such forms include, but are not limited to, antigen alone, antigen in conjunction with one or more adjuvants, antigen in association with or conjugated to a moiety, such as a hapten.

[0038] A "host cell" denotes a eukaryotic cell that has been genetically altered, or is capable of being genetically altered by administration of an exogenous polynucleotide, such as a recombinant plasmid or vector. When referring to genetically altered cells, the term refers both to the originally altered cell, and to its progeny.

[0039] An "isolated" polynucleotide, polypeptide, ganglioside, or other component, is one that is substantially free of the materials with which it is associated in nature. Substantially free means at least 50%, preferably at least 75%, more preferably at least 90%, and even more preferably at least 98% free of the materials with which it is associated in nature, other than solvent.

[0040] A "vaccine" is a pharmaceutical composition for human or animal use, which is administered with the intention of conferring the recipient with a degree of specific immunological reactivity against a particular target, or group of targets. The immunological reactivity may be desired for experimental purposes, for treatment of a particular condition, for the elimination of a particular substance, or for prophylaxis. An active vaccine is a vaccine intended to elicit an immune response in the recipient that persists in the absence of the vaccine components.

[0041] "Adjuvant" as used herein in the context of a pharmaceutical preparation is a chemical or biological agent given in combination with an antibody, polynucleotide or polypeptide to enhance its immunogenicity.

[0042] An "individual" or "subject" treated according to this invention is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, rodents, primates, and pets.

[0043] Other terms used in this disclosure are explained where they arise.

General techniques

[0044] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A

Laboratory Manual", second edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (M.J. Gait, ed., 1984); "Animal Cell Culture" (R.I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Handbook of Experimental Immunology" (D.M. Weir & C.C. Blackwell, eds.); "Gene Transfer Vectors for Mammalian Cells" (J.M. Miller & M.P. Calos, eds., 1987); "Current Protocols in Molecular Biology" (F.M. Ausubel et al., eds., 1987); "PCR: The Polymerase Chain Reaction", (Mullis et al., eds., 1994); "Current Protocols in Immunology" (J.E. Coligan et al., eds., 1991).

[0045] All patents, patent applications, articles and publications mentioned herein, both supra and infra, are hereby incorporated herein by reference.

Target antigens in psoriatic tissue

[0046] The target antigen against which a specific immune response is desired contains an epitope or antigenic determinant which is aberrantly expressed in psoriatic tissue. This means that it is present in cells of the affected tissue (or the surrounding milieu) in a manner that makes it accessible to the immune system at a level that is significantly different than in cells of the same tissue type that are not affected. The cells in which the epitope is aberrantly expressed will be resident cells of the affected area, particularly keratinocytes or living epithelial cells, rather than migrant cells. For purposes of this invention, compositions described herein may comprise an antigen or an antigenic determinant.

[0047] The aberrantly expressed antigenic determinant of interest can be of any chemical nature, including but not limited to protein epitopes, carbohydrate epitopes, and glycolipids such as gangliosides. Typically, the epitope is uniquely expressed and/or overexpressed in psoriatic tissue in a variety of different individuals, although variations in expression level may occur. Typically, the determinant will be expressed at the cell surface or present as an insoluble but accessible antigenic mass, rather than being a soluble factor. Histocompatibility Class I or Class II antigens are excluded by definition.

[0048] Antigenic determinants that are aberrantly expressed can be identified by a number of techniques known in the art. One method is to look for a particular messenger RNA transcript that is present in an unusual abundance in affected cells. Where the

determinant is a protein antigen synthesized by the cell, a suitable proxy is, for example, mRNA encoding the antigen. Where the determinant is a carbohydrate or glycolipid, a suitable proxy is, for example, mRNA encoding an enzyme involved in forming a critical antigen-specific linkage in the synthetic pathway.

[0049] Methods for determining mRNA that is in an unusual abundance in one cell than another involves preparing mRNA from both cells, and then comparing the amount of each particular mRNA between the preparations. A number of techniques for the comparison step are related in some way to subtractive hybridization. One example involves producing positive and negative cDNA, respectively, from the first and second RNA preparations, and looking for cDNA which is not completely hybridized by the opposing preparation. Subtractive hybridization is described in the standard molecular biology reference books listed earlier.

[0050] Another way of comparing production of particular messenger RNA is by differential display. This has been extensively described by Liang et al. (1992, 1995), and has since been elaborated in a number of different ways. In this technique, cDNA is prepared from only a subpopulation of each RNA preparation, and expanded via the polymerase chain reaction using primers of particular specificity. Similar subpopulations are compared across several RNA preparations for expression differences by gel autoradiography. In order to survey the RNA preparations entirely, the assay can be repeated with a comprehensive set of PCR primers. The screening strategy more effectively includes multiple positive and negative control samples (Sunday et al.). By excising the corresponding region of the separating gel, it is possible to recover and sequence the cDNA.

[0051] Antigenic determinants that are aberrantly expressed in psoriatic tissue can also be identified directly using antibodies directed against them. Psoriatic tissue or an antigen extract can be used to screen an immunoglobulin library. More conveniently, if the practitioner suspects a particular antigen of being aberrantly expressed in psoriatic tissue, an antibody specific to the antigen can be provided and used to test psoriatic tissue.

[0052] Confirmation of the overexpression can be obtained, for example, by performing a standard immunoassay on solubilized tissue extract of affected tissue, and comparing with a similar extract of unaffected tissue. More preferable are immunohistology

techniques, using the antibody specific for the suspected target as the primary antibody. A suitable aberrantly expressed determinant will result in different staining in a section from affected tissue than unaffected tissue, and will also show specificity for particular cells in the affected sample. An illustration of this is provided in Example 1.

[0053] Possible candidate antigens include any naturally occurring ganglioside antigens, antigens that are aberrantly expressed in a spectrum of different cancers, such as CEA, alpha-fetoprotein and gp-72, and antigens that are aberrantly expressed in cancers of the skin, especially but not limited to melanoma. Melanoma-associated antigens include the gangliosides GM2, GD2, GD3, and their derivatives and analogs; HMW-MAA, MPG, and gp-75. Also of interest are the Thomsen-Friedenreich (T) antigen ($\text{Gal}\beta 1-3\text{GalNAc}\alpha\text{-O-Ser}$), Tn, and sialylated Tn ($\text{New5Ac}\alpha 2-6\text{GalNAc}\alpha\text{-O-Ser}$). Also of interest is the cell surface marker CDw60.

[0054] Glycolipids suitable as target antigens include any of the monosialyl, disialyl and trisialyl gangliosides that occur naturally on cells in psoriatic tissue. Preferred target antigens are GM1, GD1a, GT1, GT2, and especially GM2, GM3, N-glycolyl GM3, GD1b, 9-O-acetyl GD2, GD3, GD3 lactone, 9-O-acetyl GD3, and GT3.

Types of therapeutic compositions used in this invention

[0055] Once a suitable target antigen is identified, a composition is designed with a view to recruiting a specific immune response in the subject to be treated against the aberrantly expressed determinant. Any type of composition capable of eliciting the desired specific immune response is suitable.

[0056] In some embodiments, the invention employs a composition comprising the target antigen, or a modified version, in an immunogenic form. In other embodiments, an effective amount of a composition is employed, wherein an effective amount is an amount sufficient to elicit the desired immune response. Accordingly, the antigen is administered in a composition such that an immune response is elicited. For example, the target antigen can be provided by obtaining an enriched fraction from a suitable tissue source or cell line, such as keratinocytes, using one of the assays described earlier to follow the desired activity. An

illustration of this is a vaccine for the Tn and sialylated Tn antigens, which can be prepared from ovine submaxillary gland mucin (O'Boyle et al.).

[0057] More typically, the target antigen is provided in synthetic form. Where the target antigen is not previously known, it is first characterized by isolation of the antigen complex, or an mRNA encoding it. Protein antigens can then be prepared by standard peptide synthesis, or by expressing a polynucleotide encoding it in a suitable host cell. Carbohydrate and other non-protein antigens are generally prepared by chemical synthesis, or by a combination of synthetic and isolation techniques. For example, use of cancer antigens as vaccine preparations is described in BE 1008391 and WO 92/19266 (CEA antigen), and in U.S. Patent No. 5,141,742 (melanoma-associated antigen p97).

[0058] Also suitable for use in immunogenic compositions to raise an anti-ganglioside response are any monosialyl, disialyl and trisialyl gangliosides, along with synthetic derivatives, including 9-O-acetyl derivatives, lactone and lactam derivatives and analogs, and episialo derivatives; many of which are referred to elsewhere in this disclosure.

[0059] Included in the practice of the invention is the application to psoriasis of ganglioside vaccines and other formulations originally designed for cancer treatment or other modes of clinical care. For example, Helling et al. and Livingston et al. describe ganglioside GD3, in the form of synthetic multiple antigenic peptides, as conjugates with albumin, KLH, or membrane proteins of *Neisseria meningitidis*, or as proteosomes. A vaccine for stimulating or enhancing production of antibodies against 9-O-acetyl GD3 is outlined in U.S. Patent No. 5,102,663. European patent application EP 0443518 outlines a cancer vaccine containing episialo complex carbohydrates, particularly epiGM3, epiGM4, or epiGM5. European patent application EP 0661061 outlines vaccine compositions for eliciting an immune response against N-glycosylated gangliosides for cancer treatment, particularly N-glycolyl GM3. PCT patent application WO 93/10134 outlines ganglioside lactam analogue derivatives that are proposed for use in a vaccine for cancer treatment. Cheresch et al. describe the biosynthesis and expression of the disialoganglioside GD2, a relevant target antigen on small cell lung carcinoma for monoclonal antibody-mediated cytotoxicity. PCT patent application WO 94/16731 outlines ganglioside-KLH conjugate vaccines with the adjuvant QS-21, using a ganglioside selected from GM2, GM3, GD2, GD3, GD3 lactone, O-

acetyl GD3, and GT3. The metabolism of these gangliosides is altered in cancers of neuroectodermal origin, including melanoma (Hamilton et al.). Progenics Pharmaceuticals Inc. is testing a GM2-KLH conjugate with the adjuvant QS-21 in a Phase III clinical trial of patients with melanoma under the product name GMK. Also being tested is a second ganglioside conjugate vaccine, MGv, comprising a combination of KLH-conjugated GD2 and GM2. The vaccine is proposed for therapy not only of melanoma, but also of colorectal cancer, lymphoma, small cell lung cancer, sarcoma, gastric cancer, and neuroblastoma (Progenics Prospectus). Any of these compositions or their active components may be adapted for psoriasis treatment in accordance with this invention.

[0060] Another illustration of a composition suitable for eliciting a specific immunological response against a target antigen in psoriasis is an anti-idiotypic moiety. This approach to immunization arises from the network theory of Jerne, involving a second antibody (Ab2) raised against a first antibody (Ab1) which in turn is specific for the target. The Ab2 is selected not only for its ability to bind Ab1 specifically, but also for its ability to stimulate a further antibody (Ab3) that cross-reacts with the target. The use of anti-idiotypes in cancer treatment is described generally in U.S. Patent No. 5,053,224.

[0061] The term “anti-idiotypic” or “anti-idiotypic moiety” as used throughout this application is defined to include not only intact antibody molecules, but any molecules comprising at least one variable region or portion of a variable region with the desired functional properties. The variable region will typically comprise a V_H - V_L pair, but may alternatively be made up of other combinations of variable chains from antibodies or T cell receptors. Variable region fragments, fusion molecules, chimeras, and humanized variants are included, so long as the requisite functional properties are retained. The variable region may be presented in any suitable form, including but not limited to intact antibody molecules, antibody fragments (such as Fab, $F(ab')_2$, and Fv), multiple antigen proteins, and various antigen-binding constructs. Examples of constructs of particular interest include fusion constructs, such as single chain variable region polypeptides (scFv), in which a single V_H - V_L pair are linked through a flexible peptide linker sequence in a manner that permits the polypeptide to fold into the three-dimensional conformation of a single variable region. Also included are diabodies, in which two variable regions are linked by a shorter linker that

prevents folding into a single variable region, but permits chains to dimerize into bivalent molecules with two V_H-V_L sites. Other constructs of interest include polymeric forms, which contain a plurality (i.e., more than one) polypeptide. Polymeric forms may be linear or branched.

[0062] The desirable characteristics of an anti-idiotypic moiety are an ability to bind an antibody specific for the target antigen (Ab1), and the ability to elicit an antibody specific for the target antigen when injected into the intended subject. As used herein, reference to an anti-idiotypic for a particular antigen (for example, an anti-idiotypic for a ganglioside) means an anti-idiotypic that elicits an active immune response specific for the particular antigen in an individual when administered in an immunogenic form.

[0063] To obtain an anti-idiotypic with the features desired, a screening process is employed. A preferred screening method involves the following steps: (1) Positive selection for antibody (or at least a molecule having a variable region) capable of binding to the Ab1; (2) Negative selection against antibody recognizing isotypic or allotypic determinants of the Ab1; (3) Positive selection for an ability to inhibit the binding of Ab1 to the target antigen; and (4) Positive selection for an ability to induce a humoral immune response against the target antigen in experimental animals. Typically, the first step involves multiple immunization of an animal with the Ab1, preparation and cloning of hybridoma cells from the immune animal, and then testing of the cells for the desired specificity on a clone-by-clone basis. However, other ways of raising or selecting antibody or antibody equivalents are also suitable. Immunocompetent phage can be constructed to express immunoglobulin variable region segments on their surface. See Marks et al., *New Engl. J. Med.* 335:730, 1996; International patent applications WO 94/13804, WO 92/01047, and WO 90/02809; and McGuinness et al., *Nature Biotechnol.* 14:1149, 1996. Phage of the desired specificity can be selected, for example, by adherence to Ab1 attached to a solid phase, and then amplified in *E. coli*. A further elaboration of the screening method and its use in preparing an anti-idiotypic for a ganglioside is provided in International Patent Application WO 96/22373.

[0064] Of particular interest are the monoclonal antibodies (and derivatives with the same immunogenic properties) with the designations 1A7, 4B5, and BEC-2. The 1A7 antibody is an anti-idiotypic for ganglioside GD2, and is described in US Patent No.

5,612,030 and International Patent Application WO 96/22373. It was deposited with the American Type Culture Collection (ATCC), now located at 10801 University Blvd., Manassas VA 20110-2209, USA, on December 28, 1994 under Accession No. HB-11786. The 4B5 antibody is an anti-idiotypic for ganglioside GD2, and is described in US Patent No. 5,653,977. The BEC-2 antibody is an anti-idiotypic for ganglioside GD3, and is described in US Patent No. 5,529,922. Also of interest are the anti-GD2 anti-idiotypes of Cheung et al., and the anti-GM3 anti-idiotypes of Yamamoto et al., one of which was recombined by Hastings et al. as a chimera with human κ constant region sequences.

[0065] Preferably, anti-idiotypic antibody 1A7 (for a functional portion thereof, as described below) is used. 1A7 was raised against the anti-GD2 monoclonal antibody designated 14G2a.

[0066] The 1A7 antibody can be prepared in several ways. It is most conveniently obtained from the hybridoma deposited with the ATCC under Accession No. HB-11786, or the progeny thereof. For example, the cells can be cultured in a suitable medium, and spent medium can be used as an antibody source. Optionally, matrix-coated channels or beads and cell co-cultures may be included to enhance growth of antibody-producing cells. For the production of large amounts of antibody, it is generally more convenient to obtain an ascites fluid. The method of raising ascites generally comprises injecting hybridoma cells into an immunologically naive histocompatible or immunotolerant mammal, especially a mouse. The mammal is optionally primed for ascites production by prior administration of a suitable composition; for example, Pristane.

[0067] Alternatively, 1A7 can be chemically synthesized in conjunction with standard methods of protein synthesis. A suitable method is the solid-phase Merrifield technique. Automated peptide synthesizers are commercially available, such as those manufactured by Applied Biosystems, Inc. (Foster City, CA).

[0068] 1A7 may also be obtained by employing routine recombinant methods such as described in Sambrook et al. (1989). For instance, using the sequences and information provided herein, a polynucleotide encoding either the 1A7 heavy or light chain can be cloned into a suitable expression vector (which contains control sequences for transcription, such as a promoter). The expression vector is in turn introduced into a host cell. The host cell is

grown under suitable conditions such that the polynucleotide is transcribed and translated into a protein. Heavy and light chains of 1A7 may be produced separately, and then combined by disulfide bond rearrangement. Alternatively, vectors with separate polynucleotides encoding each chain of 1A7, or a vector with a single polynucleotide encoding both chains as separate transcripts, may be transfected into a single host cell which may then produce and assemble the entire molecule. Preferably, the host cell is a higher eukaryotic cell that can provide the normal carbohydrate complement of the molecule. The 1A7 thus produced in the host cell can be purified using standard techniques in the art. A polynucleotide encoding 1A7 for use in the production of 1A7 by any of these methods can in turn be obtained from the hybridoma producing 1A7, or be produced synthetically or recombinantly from the DNA sequence provided herein.

[0069] Methods of antibody isolation are well known in the art. See, for example, Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. The 1A7 antibody is a mouse immunoglobulin of the IgG1 subclass, and may be isolated by any technique suitable for immunoglobulins of this isotype. Purification methods may include salt precipitation (for example, with ammonium sulfate), ion exchange chromatography (for example, on a cationic or anionic exchange column run at neutral pH and eluted with step gradients of increasing ionic strength), gel filtration chromatography (including gel filtration HPLC), and chromatography on affinity resins such as protein A, protein G, hydroxyapatite, and anti-immunoglobulin. 1A7 may also be purified on affinity columns comprising the 14G2a paratope; for example, in the form of a purified Ab1 or Ab3. Preferably, 1A7 is purified from BALB/c ascites using Protein-A-CL-SEPHAROSE™ 4B chromatography followed by chromatography on a DEAE-SEPHAROSE™ 4B ion exchange column.

[0070] Alternatively, an active portion (see above under description of “anti-idiotypic” or “anti-idiotypic moiety”) of 1A7 may be used, which comprise a portion of or an entire variable region of 1A7. Examples of variable region (whether intact or fragments) constructs have been provided above. Figures 2 and 3 provide the polypeptide sequences of the light and heavy chain variable regions as well as the polynucleotide sequences encoding the

variable regions. Preparation of these 1A7 polypeptides employs standard techniques, such as recombinant techniques, in the art.

[0071] 1A7 polypeptides can be produced by proteolytic or other degradation of 1A7, by recombinant methods (i.e., single or fusion polypeptides) as described above or by chemical synthesis. 1A7 polypeptides, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, a 1A7 polypeptide could be produced by an automated polypeptide synthesizer employing the solid phase method.

[0072] Preferably, the polypeptides are at least partially purified from other cellular constituents. Preferably, the polypeptides are at least 50% pure. In this context, purity is calculated as a weight percent of the total protein content of the preparation. More preferably, the proteins are 50-75% pure. More highly purified polypeptides may also be obtained and are encompassed by the present invention. For clinical use, the polypeptides are preferably highly purified, at least about 80% pure, and free of pyrogens and other contaminants. Methods of protein purification are known in the art and are not described in detail herein. Alternatively, if a 1A7 polypeptide(s) is expressed in a suitable storage medium, such as a plant seed, the 1A7 polypeptide need not be purified and could even be administered without purification. Fiedler et al. (1995) *Biotechnology* 13:1090-1093.

[0073] 1A7 polypeptides can be obtained from intact 1A7, which can in turn be isolated from the hybridoma ATCC Accession No. HB-11786 producing 1A7, which is described in co-owned U.S. Patent 5,612,030 and International Patent Application WO 96/22373. Techniques of isolating antibodies from hybridomas are well known in the art. See, e.g., Harlow and Lane (1988). Once intact 1A7 is obtained, 1A7 polypeptides can be obtained by degradation of intact 1A7, by using, for example, proteolytic enzymes (proteinases). Examples of proteolytic enzymes include, but are not limited to, trypsin, plasmin, and thrombin. Intact 1A7 can be incubated with one or more proteinases, or the digestions can be performed sequentially. The nature and extent of the proteolytic cleavage will depend upon the desired polypeptide length as well as the enzymes used. These techniques are well known in the art. Alternatively, or in addition, intact 1A7 can be treated with disulfide reducing agents to disassociate the molecule.

[0074] 1A7 polypeptides can also be made by chemical synthesis using techniques known in the art. 1A7 polypeptides can also be made by expression systems, using recombinant methods. The availability of 1A7 polynucleotides encoding 1A7 polypeptides permits the construction of expression vectors encoding intact 1A7, functionally equivalent fragments thereof, or recombinant forms of 1A7. A polynucleotide encoding the desired 1A7 polypeptide, whether in fused or mature form, and whether or not containing a signal sequence to permit secretion, may be ligated into expression vectors suitable for any convenient host. Both eukaryotic and prokaryotic host systems can be used. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification or isolation of the polypeptides expressed in host systems can be accomplished by any method known in the art. For example, cDNA encoding intact 1A7 or a fragment thereof can be operatively linked to a suitable promoter, inserted into an expression vector, and transfected into a suitable host cell. The host cell is then cultured under conditions that allow transcription and translation to occur, and the desired polypeptide is recovered. Other controlling transcription or translation segments, such as signal sequences that direct the polypeptide to a specific cell compartment (i.e., for secretion), can also be used. Examples of prokaryotic host cells are known in the art and include, for example, *E. coli*. Examples of eukaryotic host cells are known in the art and include yeast, avian, insect, plant, and animal cells such as COS7, HeLa, CHO and other mammalian cells.

[0075] For scFv fragments, light and/or heavy chain variable regions are linked using a short linking peptide. Bird et al. (1998) *Science* 242:423-426. An example of a linking peptide is (GGGS)₃, which bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used. Bird et al. (1988). Usually the linkers are selected to have little to no immunogenicity. For asymmetrical linkers, the scFvs can be assembled in any order. Generally, the entire variable regions are included in the scFv, which may be produced either recombinantly or synthetically.

[0076] Another illustration of a composition suitable for eliciting a specific immunological response against a target antigen in psoriasis is an expression vector encoding a polypeptide which is used in an immunogenic composition. The polypeptide encoded by

the vector is either one comprising the antigenic determinant that is aberrantly expressed in psoriatic tissue, or else an anti-idiotype for the aberrantly expressed determinant. The encoding region is linked in the vector to suitable transcription and translation control elements that permit the encoding region to be expressed in the intended subject upon administration.

[0077] Vaccines made up of naked polynucleotides are generally described in Tang et al. (1992) *Nature* 356: 152-154. Viral vectors are also suitable, including by way of example, vectors based on herpes viruses, hepadna viruses, Sindbis virus, pseudotype retroviral vectors, adenovirus, adeno-associated virus. Where the encoding region encodes an anti-idiotype, of particular interest are vectors based on vaccinia virus that can be used in vaccine preparations (Moss (1991) *Science* 252:1662-1667). Such vectors can be constructed, for example, by homologous recombination of vaccinia plasmids and wild-type WR strain of vaccinia virus using CV-1 cells, according to the procedure of Mackett et al. (DNA Cloning, Vol. II, D.M. Glover, ed., IRL Press 1985).

Formulation of therapeutic compositions

[0078] The preparation of pharmaceutical compositions is conducted in accordance with generally accepted procedures for the preparation of pharmaceutical preparations. See, for example, *Remington's Pharmaceutical Sciences 18th Edition* (1990), E.W. Martin ed., Mack Publishing Co., PA. Depending on the intended use and mode of administration, processing optionally includes sterilizing, mixing with appropriate non-toxic and non-interfering components, dividing into dose units, or enclosing in a delivery device.

[0079] Protein vaccines used in this invention typically comprise an adjuvant, which may be the same as or in addition to the excipient or carrier. Examples of adjuvants include but are not limited to aluminum hydroxide, alum, QS-21 (U.S. Patent No. 5,057,540), DHEA (U.S. Patent Nos. 5,407,684 and 5,077,284) including its precursors and modified forms (e.g., DHEA-S, the sulfonated form of DHEA), β 2 microglobulin (WO 91/16924), muramyl dipeptides, muramyl tripeptides (U.S. Patent No. 5,171,568), monophosphoryl lipid A (U.S. Patent No. 4,436,728; WO 92/16231) and its derivatives, such as various forms and

generations of DETOX™ and BCG (U.S. Patent No. 4,726,947). Other suitable adjuvants are aluminum salts, squalene mixtures (SAF-1), muramyl peptide, saponin derivatives, mycobacterium wall preparations, mycolic acid derivatives, nonionic block copolymer surfactants, Quil A, cholera toxin B subunit, polyphosphazene and derivatives, and immunostimulating complexes (ISCOMs) such as those described by Takahashi et al. (1990) Nature 344:873-875. For veterinary use and for production of antibodies in animals, complete and incomplete Freund's adjuvant can be used. A preferred vaccine composition for peptides and anti-idiotypes or peptide derivatives thereof is prepared by mixing with aluminum hydroxide and incubated to about 48°C for about 30 min.

[0080] Especially preferred adjuvants include QS-21 and RIBI™PC. The QS-21 molecule consists of a triterpene glycoside with the general structure of a quillaic acid 3,28-O-bis glycoside. There are two structural isomers designated V-1 and 2-1 at a typical ratio of ~ 2:1, both of which have adjuvant activity. QS-21 has been shown to stimulate a response against T-dependent antigens and unconjugated T-independent antigens. QS-21 also augments the induction of MHC Class I cytotoxic T lymphocytes to subunit antigen vaccines, as well as antigen-specific cellular proliferation. Preclinical trials confirm its safety and efficacy at 100 µg/dose. QS-21 is supplied by Aquila Biopharmaceutical, Inc. in Worcester, MA.

[0081] The composition can optionally also contain other active medicinal agents, and/or non-active ingredients such carriers, and auxiliary substances such as wetting or emulsifying agents, and pH buffering agents. Additives of particular interest are adjuncts that enhance the immunogenic effect, such as mitogens or stimulatory cytokines. Of particular interest as adjunctive agents for anti-idiotype compositions are interleukins, particularly IL-2. The composition is typically formulated in liquid form, but can also be freeze-dried for reconstitution by hydration.

[0082] The route of administration is selected according to the formulation of composition and the intended effect. In the more usual embodiments of this invention, the composition is formulated and administered so as to stimulate a systemic response in the subject. With this in view, possible routes of administration include intracutaneous, subcutaneous, intramuscular, intraperitoneal, intradermal, oral, intranasal, intradermal, and

intrapulmonary (i.e., by aerosol). Protein vaccines of this invention for human use are typically administered by a parenteral route, most preferably subcutaneous. A series of injections is preferably given at different subcutaneous sites.

[0083] In other embodiments, the composition is formulated and administered to produce a local effect (i.e., is formulated for topical administration). These compositions are generally used to boost at an affected site a response in an individual where a systemic immunological response against the target antigen has already successfully been induced, although pre-generation of a systemic immune response is not required. In these embodiments, the composition is generally in the form of a cream or gel, or other combination of the active ingredient and a readily absorbable or evaporable excipient. The composition is then administered on the skin at the affected site. Accordingly, the invention provides topical formulations of any of the target antigen (antigenic determinants) described herein, such as gangliosides, anti-idiotypic moieties (i.e., anti-idiotypic moieties, including anti-idiotypic antibodies, for any of the gangliosides described herein), and the expression vectors described herein. Preferably, a topical formulation comprises 1A7. Examples of suitable topical formulations, which are well known in the art, include ointments, creams, and gels.

[0084] In certain embodiments of the invention, the composition is specially designed for psoriasis, comprising a formulation and dose that is designed especially to maximize management of the psoriatic condition. In other embodiments, the composition is formulated in a similar fashion as it would be for use in another type of therapy, such as for cancer treatment, that shares a common objective, such as the eliciting of an immunological response against an antigen that is aberrantly expressed in either condition.

[0085] Preferably, compositions useful for treating psoriasis according to the present invention are accompanied by written instructions as part of the packaging or in a product insert. The written instructions can simply indicate that psoriasis (in any one of its many forms) is a suitable indication for the use of the composition. Optimally, the instructions will also indicate suitable subjects and conditions, the recommended route of administration, timing, and dosage, contraindications, potential side effects, and expected benefit.

[0086] The invention accordingly provides a composition for use (or use of any of the composition compositions described herein) in the preparation of a medicament for use in the treatment of psoriasis. These compositions comprise any of the embodiments described herein. In one embodiment, the invention provides use of a component selected from the group consisting of i) a ganglioside; ii) an anti-idiotypic moiety for a ganglioside; and iii) an expression vector encoding an anti-idiotypic for a ganglioside in the manufacture of a medicament for the treatment of psoriasis. The psoriasis to be treated may be any of guttate psoriasis, pustular psoriasis, plaque-type psoriasis, psoriatic arthritis, and/or chronic plaque psoriasis. The ganglioside may be any of GM2, GM3, GD1B, GD2, 9-O-acetyl GD2, GD3, GD3 lactone, 9-O-acetyl GD3, and GT3. The anti-idiotypic moiety may be any of 1A7, 4B5, or BEC-2, and is preferably 1A7.

Use of therapeutic compositions

[0087] Patients suitable for treatment according to this invention have clinical or histological features of psoriasis, particularly guttate psoriasis, pustular psoriasis, plaque-type psoriasis, or psoriatic arthritis. The compositions can also be given to patients who have no outward signs of psoriasis, but are at risk for developing the disease especially in its more severe manifestations (because of a genetic predisposition, family history or previous manifestations), although this is less typical. Since the effectiveness of this invention is believed to involve a host immunological response against the target antigen, the therapeutic compositions are predicted to be more effective when the individual is not immunodeficient or immunocompromised due to a genetic abnormality, infection, or by chemical treatment.

[0088] The amount of the immunogenic substance administered at one time is selected with a view to clinical safety, and to achieve the initial desired immunological and clinical result within a few administrations. The range of effective concentrations for protein immunogens is generally about 10 µg to 20 mg, and typically 200 µg to 10 mg, with the tendency towards higher values where the immunogenic determinant is a proportionately smaller part of the protein. The range of effective concentrations for polynucleotide vaccines is generally about 10 µg to 500 µg of nucleic acid, typically about 50 to 100 µg. Since

clinical efficacy is believed to correlate with the extent of the specific immunological response obtained, a dose that is clinically effective can in principle be predicted by determining an immunogenic dose in an animal model, and then scaling the dose appropriately for human use. Administrations are typically conducted on a weekly or biweekly basis until there is evidence of an immunological or clinical response. Administration can then be continued on a less frequent basis, such as biweekly, monthly, or bimonthly as appropriate.

[0089] Treatment according to this description can optionally be combined with other regimens focused on clinical symptoms, including but not limited to local treatment with topical steroids, topical calcipotriol, or ultraviolet light; or systemic treatment with methotrexate, etretinate or cyclosporine. Parallel treatment can also be conducted with a view to activating the immune system to make it more responsive to the vaccine, such as simultaneous administration of a mitogen or cytokine. In one example, IL-2 is injected at a collateral site at a dose of about 1.5 to $15 \times 10^6 \text{ U m}^{-2} \text{ day}^{-1}$, either throughout the priming phase of vaccine treatment, or as a pulse given a few consecutive days on a biweekly schedule, or any reasonable variation.

[0090] Clinical response is measured around about the time of administration and on regular follow-up. Immunological response can also be measured, if desired, by collecting periodic blood samples for analysis.

[0091] Presence of antibody activity (for example, against a particular ganglioside or a particular anti-idiotype) can be determined by standard immunoassay of serum or plasma samples from the treated subject. For anti-idiotype activity, the sample is preincubated with autologous immunoglobulin or adsorbed on a suitable affinity resin to remove antibody activity against isotypic and allotypic determinants. In one assay method, the sample is incubated in a microtiter plate well previously coated with the target antigen; the well is washed, and then the reaction is developed with an isotopically or enzymatically labeled anti-immunoglobulin reagent. Results are compared with those using preimmune serum or serum from subjects immunized with an unrelated antigen. Specificity can also be measured by Western blot. The antigen is separated by electrophoresis over a polyacrylamide gel, blotted onto nitrocellulose, and then developed with the sample. In a third example, the sample is

incubated with cells that do or do not express the antigen of interest, and then developed using a fluorescently tagged anti-immunoglobulin. Staining frequency and intensity can then be measured by FACS analysis. A model cell line is one expressing GD2 is M21/P6. In a fourth example, the sample is overlaid onto a histological sample from a psoriatic lesion, as may be taken from the treated subject, and then developed with an enzyme-labeled anti-immunoglobulin. This is illustrated in Example 3. The nature of the response in the sample can be further characterized in any of these assays by competition with an antibody with known activity for the target antigen. A model antibody for GD2 is monoclonal antibody 14G2a.

[0092] Complement mediated cytotoxicity (CMC) can be measured, if desired, using a cell line that expresses the target antigen. The cell line is labeled with a cytosolic marker, such as ^{51}Cr . The assay is conducted by adding and incubating a sample suspected of containing antibody. Complement is added in a suitable form, such as guinea pig serum pre-adsorbed with the cell line. After a suitable incubation period at 37°C , extent of ^{51}Cr release is then measured and compared with that of unopsonized control cells. ^{51}Cr labeled target cells can also be used to measure antibody-dependent cell-mediated cytotoxicity (ADCC) in the sample, by supplying human peripheral blood mononuclear cells (PBMC) from normal subjects as effector cells at a ratio of effector:target cells of about 100:1.

[0093] A cell mediated immune response in a subject can be measured by isolating PBMC from a blood sample of the treated subject, collected into heparinized tubes, and separated on a suitable gradient such as Ficoll-HypaqueTM. To measure T cell proliferation (a general indicator of T cell activation against the antigen), the cells are incubated with a range of concentrations of the target antigen. A non-specific mitogen such as PHA serves as a positive control; incubation with an unrelated antigen serves as a negative control. After incubation of the PBMCs for an appropriate period (typically 5 days), [^3H]thymidine incorporation is measured, and the proliferating cells can be further characterized by flow cytometry using cell type specific markers. Cytotoxic T cell response can be measured after a period of stimulation by presenting with ^{51}Cr labeled antigen-bearing target cells.

[0094] Clinical outcome is followed over the course of therapy for a therapeutic effect and undesirable side-effects, as illustrated in Example 4. Ideally, the compositions promote

resolution of the clinical features, but stabilization of the condition is a satisfactory outcome. Progression or regression of the disease is followed according to the particular clinical manifestations of the original presentation, and typically includes the number and size of psoriatic lesions, and the extent of total body surface area that is involved. Status can also be measured at the microscopic level as the proportion of epidermal cell proliferation or the proportion of cells expressing target antigen.

[0095] The selection of the target antigen (or antigenic determinant) and the formulation of the immunogenic composition can be adjusted by the manufacturer, and the exact dose and timing for the administration can be adjusted by the administering physician, without departing from the spirit of the invention.

[0096] The invention also provides methods for preparing a composition for use in the treatment of psoriasis, comprising raising an anti-idiotypic antibody against a monoclonal antibody that binds an antigenic determinant that is aberrantly expressed in psoriatic tissue, wherein the anti-idiotypic antibody is capable of eliciting an immunological response in a human against the antigenic determinant. Alternatively, the methods comprise preparing an immunogenic composition comprising the aberrantly expressed determinant.

Screening methods of the invention

[0097] The invention also provides methods of screening immunogenic compositions for use in treatment of psoriasis in humans.

[0098] In some embodiments, the methods comprise the steps of (a) administering to a plurality of human subjects having psoriasis an immunogenic composition comprising an immunogenic form of i) an antigenic determinant that is aberrantly expressed in psoriatic tissue; ii) an monoclonal anti-idiotypic antibody for an antigenic determinant that is overexpressed in psoriatic tissue; or iii) an expression vector encoding either i) or ii); and (b) correlating the progression of the psoriasis in the human subjects treated in step (a) in relation to that in untreated human subjects having psoriasis, with the effectiveness of the immunogenic composition.

[0099] In other embodiments, the screening methods comprise the following steps: a) administering to a plurality of human subjects having psoriasis an immunogenic composition, comprising an immunogenic form of: i) an antigenic determinant that is aberrantly expressed in psoriatic tissue; ii) a monoclonal anti-idiotypic antibody for an antigenic determinant that is aberrantly expressed in psoriatic tissue; or iii) an expression vector encoding either i) or ii); and b) determining the extent of an immunological response against the antigenic determinant in each subject treated in step a); and c) correlating the progression of the psoriasis in relation to the extent of the immunological response against the antigenic determinant in each subject with the effectiveness of the immunogenic composition.

[0100] In any of these screening embodiments, the antigen that is aberrantly expressed in psoriatic tissue may preferably be a ganglioside, more preferably GD2.

Kits of the invention

[0101] The invention also provides kits comprising a) a composition packaged in a container and comprising an immunogenic form of a component selected from the group consisting of: i) a ganglioside; ii) an anti-idiotypic antibody for a ganglioside; and iii) an expression vector encoding an anti-idiotypic antibody for a ganglioside; and b) written instructions for using the composition in the treatment of psoriasis. Preferably, the component is 1A7.

[0102] The examples presented below are provided as a further guide to a practitioner of ordinary skill in the art, and are not meant to be limiting in any way.

EXAMPLES

Example 1: Demonstration of ganglioside antigen in psoriasis tissue

[0103] In this experiment, histology sections from psoriasis lesions were screened for the presence of aberrantly expressed antigens that would be suitable targets for an active vaccination strategy.

[0104] Skin-punch biopsies were obtained from patients having benign *Psoriasiform Dermatitis*. Paraffin blocks from three samples were cut into 5 μ m cross-sections through the dermal layers. The sections were fixed onto slides and deparaffinized by a series of graduated washes of decreasing ethanol content. Once equilibrated into phosphate-buffered saline, pH 7.4, the sections were blocked against non-specific binding by overlaying with 10% normal rabbit serum that had been heat-inactivated.

[0105] After washing, the sections were overlaid with one of a panel of primary antibodies purified by anion exchange chromatography as required, and diluted to 50 μ g/mL. The section was incubated with the primary antibody at room temperature for about an hour, and then rewashed. The section was next overlaid with biotinylated rabbit anti-mouse immunoglobulin as secondary antibody, incubated, and washed. The sections were developed using a streptavidin-peroxidase conjugate, followed by substrate.

[0106] The panel of primary antibodies consisted of the following:

- Monoclonal antibody 14G2a, grown from a hybridoma cell line obtained from the Scripps Research Institution. 14G2a has been subtyped as an IgG2ak. 14G2a is specific for the ganglioside antigen GD2, and does not bind to gangliosides GM1, GM2, GM3, GD3, or GT1b. The ability of 14G2a to bind GD2 in fixed tissue was confirmed by immunoperoxidase staining of paraffin blocks of human melanoma tissue. 14G2a also specifically binds the GD2 positive melanoma cell line M21/P6 when used as the primary antibody in FACS analysis.
- Monoclonal antibody 8019, produced from the hybridoma obtained from the American Type Culture Collection (ATCC, Rockville MD). 8019 has been subtyped as an IgG1 κ , and is specific for carcinoembryonic antigen (CEA). The ability of 8019 to bind CEA in fixed tissue was confirmed by immunoperoxidase staining of paraffin blocks of human colon cancer samples. 8019 also specifically binds the CEA positive colon cancer cell line LS174-T when used as the primary antibody in FACS analysis.
- Monoclonal antibody MC-10, also designated BrE-1, is specific for human milk fat globule (HMFG), an antigen aberrantly expressed in breast cell carcinomas. MC-10 has been subtyped as an IgG2bk. The ability of MC-10 to bind HMFG in

fixed tissue sections was confirmed by immunoperoxidase staining of paraffin blocks of human breast cancer samples. MC-10 also specifically binds the HMFG positive breast cancer cell lines MCF-7 and SKBR3 when used as the primary antibody in FACS analysis.

- A monoclonal mouse IgG2b of unknown specificity, purchased from Sigma Chemical Co., serving as a negative control.

[0107] Staining intensity was graded on a scale of (–) to (++++). The following results were obtained:

TABLE 1: Immunohistochemical staining for aberrantly expressed antigen			
Primary Antibody	Staining Intensity		
	Sample 1	Sample 2	Sample 3
14G2a	+++	+++	+++
8019	++++	++++	++++
MC10	±	±	±
mouse IgG2b control	–	–	–
PBS	–	–	–

[0108] Both 14G2a and 8019 antibodies gave strong staining in all samples tested. The staining pattern was consistent with specific staining for affected tissue, since only dermal layers were stained. Monoclonal antibody MC10 was essentially negative.

[0109] These results indicate that both GD2 and CEA are candidate targets for a vaccine strategy against psoriasis.

Example 2: Eliciting an anti-GD2 response in humans using an anti-idiotypic

[0110] Hybridoma cells expressing monoclonal anti-idiotypic antibody 1A7 (U.S. Patent No. 5,612,030) were used for the production of ascites fluid. 9.7 g of purified antibody was prepared by TSD BioServices under GMP-conditions. The regulatory testings on the antibody preparation were completed according to FDA guidelines. This example

describes how the antibody is tested in a human clinical study to verify safety and demonstrate its ability to elicit an anti-GD2 response in humans.

[0111] Patients are immunized with 1A7 antibody mixed with 100 µg of QS-21 adjuvant. Patients are randomized to one of the four dose levels. The total number of patients is between about 12 and 32. Injections are given biweekly for four total doses, or until an immune response is observed. Therapy continues with monthly injections until tumor progression is found. Patients are monitored carefully for anaphylaxis, serum sickness, and other potential side effects.

[0112] Periodic blood samples are obtained to determine the effect on hematopoietic cells as well as renal and hepatic function. All patients entered into the study undergo leukapheresis prior to the first immunization (pre-therapy). In addition, blood samples are obtained prior to each injection of 1A7 to determine serum levels of Ab3 and Ab1' antibodies and cytotoxic T cell responses. The specificity of the humoral responses is confirmed by immune flow cytometry, radioimmunoassay, and dot blot analysis. Antiglobulin responses to the murine antibody is tested by sandwich radioimmunoassay. Sera are also tested for an ability to inhibit the binding of anti-GD2 mAb to GD2 antigen. The immune profile of patients is further assessed by testing the proliferative response of patient's lymphocytes to anti-idiotypic antibody, purified GD2 antigen, and irradiated tumor cells and the cytotoxicity of patient's lymphocytes for GD2-positive HLA-matched cell lines or autologous tumor cells (where possible).

[0113] What follows are the results from seven participating in the study over a sufficient period to evaluate the presence of an immunological response. Each patient was immunized with 1 mg, 2 mg, 4 mg, or 8 mg of antibody 1A7 in QS-21 on a biweekly schedule. For the first few patients in the study, the first 2 to 4 doses were given intramuscularly, and periodic serum samples were collected to determine the presence of human anti-mouse (HAMA) activity and anti-1A7 activity. Titers were low, and it was decided to continue the course of immunization subcutaneously. All patients seroconverted positive with respect to both HAMA and anti-1A7, as determined by immunoassay. The response comprised specific Ab3 activity, as demonstrated by the ability of each serum to inhibit the binding of radiolabeled 1A7 to solid-phase linked 14G2a (Ab1). None of the

patients have yet shown objective clinical responses related to their cancer. However, three of the seven patients (270+ to 510+ days) have stable disease and continue on vaccine therapy.

[0114] The results demonstrate that administration of 1A7 as a pharmaceutical composition with the adjuvant QS-21 is very well tolerated up to a dose of 8 mg, and is highly effective in generating an anti-GD2 response.

[0115] To investigate the nature of the response further, anti-1A7 antibody was affinity purified from the sera of four of the patents. First, each sample was passed over a column of 14G2a antibody, eluted with a glycine buffer (pH ~ 2.5), and exchanged into PBS. Next, HAMA activity that was not Ab3 was depleted by negative selection on a mouse immunoglobulin adsorbant. The amount of specific anti-1A7 (Ab3) obtained was as follows: Patient 1 (administered 1 mg 1A7 per dose), yield 0.67 mg Ab3 from 10 mL serum. Patient 2 (administered 2 mg 1A7 per dose), yield 1.32 mg Ab3 from 10 mL serum. Patient 3 (administered 4 mg 1A7 per dose), yield 1.71 mg Ab3 from 10 mL serum. Patient 4 (administered 4 mg 1A7 per dose), yield 0.73 mg Ab3 from 10 mL serum. This indicates that a substantial amount of Ab3 is produced as a result of administering 1A7 at any of the doses tested, and apparently is in molar excess of antigen in the circulation.

[0116] The affinity and specificity of the response to GD2 was further confirmed by using the affinity purified Ab3 in several of the assay systems described earlier. In one test, an assay plate was coated with ganglioside GD2 or GD3, overlaid with purified Ab3, and then developed with alkaline phosphatase labeled anti-immunoglobulin. The results showed that each patient's response comprises the production of anti-GD2 antibody (Ab1'), but not anti-GD3 antibody. In another test, an assay plate was coated with GD2, overlaid with purified Ab3, and then developed with isotype-specific anti-immunoglobulin reagents. The anti-GD2 response was apparently a mature response comprising both IgG and IgM, with IgG predominating.

[0117] Inhibition titration experiments were conducted using purified Ab3 from three different patients. In one test, an assay plate was coated with ganglioside GD2, and varying amounts of purified Ab3 were tested for the ability to inhibit the binding of radiolabeled 14G2a (Ab1). The half-titration point for each Ab3 was comparable to that of unlabeled

14G2a. In another test, varying amounts of purified Ab3 were tested for their ability to inhibit the binding of radiolabeled 14G2a to the GD2-expressing murine lymphoma cell line EL4. The results indicated that the Ab3 induced by administration of 1A7 competes for binding to GD2 aberrantly expressed by model target cells.

Example 3: Demonstration of antibody to psoriasis-associated antigen in the serum of immunized human subjects

[0118] Ab3 affinity purified from the sera of human patients treated with an anti-idiotypic antibody were tested for the presence of circulating antibody against a psoriasis associated antigen.

[0119] Patients were treated with monoclonal anti-idiotypic 1A7 (an anti-idiotypic for GD2), or with anti-idiotypic 3H1 (an anti-idiotypic for CEA). Treatment of subjects with 1A7 is described in Example 2. The preparation and use of 3H1 is described in PCT patent applications WO 96/20219 and WO 96/20277.

[0120] After a course of multiple immunizations on a biweekly immunization schedule, the respective Ab3 present in were prepared by passing immune sera over an affinity column made of the immunizing antibody, and recovering the bound fraction. As a negative control, normal or preimmune human IgG was prepared by passing serum of an unimmunized subject over a Protein G column.

[0121] The purified IgG fractions were then tested for their ability to specifically stain affected psoriasis tissue, using the immunohistochemical methods described in Example 1.

The results are shown in the following Table:

TABLE 2: Tissue staining by antibody present in the sera of immunized patients				
Primary Antibody	Staining Intensity			
	Sample 1	Sample 2	Sample 3	Sample 4
14G2a (Ab1 for 1A7; positive control)	+++	+++	+++	+++
Ab3 from patients treated with 1A7	++++	++++	++++	++++
normal human IgG	—	—	—	—
Ab3 from patients treated with 3H1	—	—	—	—

[0122] The results show that treatment of human patients with monoclonal antibody 1A7 successfully elicits an active immune response that is specific for a target antigen on affected psoriasis tissue. The target antigen is shared between different psoriasis patients, and (in view of the known immunogenic properties of the 1A7 antibody) most probably is GD2. Specificity is confirmed by two additional observations: a) the staining pattern with Ab3 from the 1A7 treated subject showed staining of the affected skin cells but not surrounding muscle fibers; b) anti-idiotypic antibody 3H1 raises a specific Ab3 response, but the response was apparently *not* specific for psoriasis tissue.

Example 4: Treating psoriasis by eliciting an active anti-ganglioside immunological response

[0123] Psoriasis afflicts approximately 1 to 2 percent of the adult population in various forms. Patients with extensive plaque psoriasis and pustular psoriasis frequently require systemic therapy. Topical steroids, topical calcipotriol, or ultraviolet light may not give adequate control. The currently used systemic medications such as methotrexate, etretinate and cyclosporine are sometimes effective, but associated with significant side effects.

[0124] A patient with advanced psoriasis was administered monoclonal antibody 1A7 as part of a clinical trial for the treatment of melanoma. After leaving the trial, the patient returned to the attending physician (Dr. K. Foon) and reported that his psoriatic systems,

which had previously been persistent, had improved. His case history and results of the immunological tests were reviewed, and it was discovered that the resolution of the symptoms correlated with the stimulation of an anti-GD2 immune response (as detected in serum) during his treatment with 1A7 in the melanoma trial. Subsequently, psoriasis tissue samples from several patients were evaluated by immunohistochemistry (Example 1), and all were positive for an antigen recognized by anti-GD2 antibody. Antibody present in the serum of several patients immunized with the GD2 anti-idiotype 1A7 was isolated, and found to react with a target antigen on psoriatic tissue (Example 3).

[0125] This example provides a method for testing the efficacy of raising an anti-GD2 immunological response in the treatment of psoriasis.

[0126] Fifteen patients who have been diagnosed with severe *chronic plaque psoriasis*, and who have had an unsatisfactory response to a prior systemic therapy, topical steroids or ultraviolet light are treated. Eligibility criteria also include absence of active infection or blood-born disease, adequate renal and hepatic function tests, granulocyte count $\geq 1000 \text{ mm}^{-3}$, platelets $> 100,000 \text{ mm}^{-3}$, absence of allergy to mouse protein, and ability to provide informed consent. Subjects are pre-screened for the presence of GD2 antigen on keratinocytes in psoriatic lesions by immunohistology, as exemplified in Example 1.

[0127] **Figure 1** shows the scheme of treatment. The admitted patients are treated with 2 mg of 1A7, mixed with 100 μg of the adjuvant QS-21. Four injections are given initially, one every other week, and then injections are given monthly. Treatment is stopped if there is a significant adverse event, if there is substantial advancement of the disease, or if there is no significant effect after six months. Patients who show advancement of the disease are followed for an additional six months after the cessation of treatment. Patients with stable or improved disease six months after initiation of treatment receive further treatment for an additional six months, followed by another evaluation.

[0128] During the course of the trial, patients are monitored for toxicity. Periodic blood samples are taken to determine any effect on hematopoietic cells, renal and hepatic function, LDH, and uric acid. The most likely side effects are local skin reaction, fever, chilling, and sweating, seldom requiring therapy and persisting for only a few hours. Anti-pruritics are used where needed. Allergic reactions are treated symptomatically with

diphenhydramine or hydroxyzine. Bronchospasm and anaphylaxis, if they occur, are treated with epinephrine and supportive care, and result in removal of the patient from the treatment regimen. In view of previous tests of 1A7 in cancer patients (Example 2), major toxicity is not anticipated.

[0129] Periodic blood samples are also taken to follow the immunological response in each patient. The presence of antibodies against 1A7 (the Ab3 response) and against GD2 (the Ab1' response) is measured by standard plate-binding immunoassay. Serum or isolated Ab3 is also tested for binding to GD2 expressed on tumor cell lines or psoriatic tissue. Antibody-dependent cellular cytotoxicity, T cell proliferative activity, or cytotoxic T cell activity may also be measured according to the procedures described elsewhere in this disclosure. Induction of both humoral and cellular anti-GD2 activity is anticipated in the majority of treated subjects after four or more injections with the 1A7 vaccine.

[0130] Results are determined as follows. Symptoms are graded according to the Psoriasis Area and Severity Index. Severity is graded on a numerical scale (0 = none; 1 = slight; 2 = moderate; 3 = severe), taking into account erythema, scaling, and induration. The extent is based on total body surface involvement (trunk = 35%; legs = 35%; arms = 20%; head and neck = 10%). Progression is then graded between -1 and +3 (-1 = worse; 0 = stable; +1 = minimal improvement; +2 = definite improvement; +3 = clearing). Clinical experience is that without treatment, the disease will typically progress, involving more surface or having plaques with more erythema and induration. The treatment is considered to be successful if there is substantial improvement or stabilization of the disease, compared to the typical course in patients not treated with the vaccine, and initially presenting with similar symptoms and clinical experience.

[0131] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent to those skilled in the art that certain changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention, which is delineated by the appended claims.

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